09/509306

# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE
in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>23 February 2001 (23.02.01) | |
| **International application No.**<br>PCT/NZ98/00145 | **Applicant's or agent's file reference**<br>23624 MRB |
| **International filing date** (day/month/year)<br>25 September 1998 (25.09.98) | **Priority date** (day/month/year)<br>26 September 1997 (26.09.97) |
| **Applicant**<br>REID, Ian, Reginald et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

22 April 1999 (22.04.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>Ingrid Aulich |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

Form PCT/IB/331 (July 1992)

NZ9800145

# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

## PCT

### NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>01 June 1999 (01.06.99) | |
| **International application No.**<br>PCT/NZ98/00145 | **Applicant's or agent's file reference**<br>23624 MRB |
| **International filing date** (day/month/year)<br>25 September 1998 (25.09.98) | **Priority date** (day/month/year)<br>26 September 1997 (26.09.97) |

**Applicant**

REID, Ian, Reginald et al

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

22 May 1999 (22.05.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [ ] was

[X] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

**The International Bureau of WIPO**
**34, chemin des Colombettes**
**1211 Geneva 20, Switzerland**

Facsimile No.: (41-22) 740.14.35

Authorized officer

Lazar Joseph Panakal

Telephone No.: (41-22) 338.83.38

2645841

Form PCT/IB/331 (July 1992)

# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

CORRECTED VERSION

PCT

## NOTIFICATION OF ELECTION
(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>08 February 2000 (08.02.00) | |
| **International application No.**<br>PCT/NZ98/00145 | **Applicant's or agent's file reference**<br>23624 MRB |
| **International filing date** (day/month/year)<br>25 September 1998 (25.09.98) | **Priority date** (day/month/year)<br>26 September 1997 (26.09.97) |
| **Applicant**<br>REID, Ian, Reginald et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

22 April 1999 (22.04.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>Catherine Massetti |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

3094325

Form PCT/IB/331 (July 1992)

Copy for the Elected Office (EO/US)

PCT/NZ98/00145

3:41

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

BENNETT, Michael, Roy
Russell McVeagh West-Walker
Mobil of the Park
P.O. Box 1344
157 Lambton Quay
Wellington 6001
NOUVELLE-ZÉLANDE

Date of mailing (day/month/year)
15 February 2000 (15.02.00)

Applicant's or agent's file reference
23624 MRB

International application No.
PCT/NZ98/00145

**IMPORTANT NOTIFICATION**

International filing date (day/month/year)
25 September 1998 (25.09.98)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
BENNETT, Michael, Roy
Russell McVeagh West-Walker
The Todd Building
Level 5
171-177 Lambton Quay
Wellington 6001
New Zealand

State of Nationality

State of Residence

Telephone No.
64 4 499 9058

Facsimile No.
64 4 499 9306

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
BENNETT, Michael, Roy
Russell McVeagh West-Walker
Mobil of the Park
P.O. Box 1344
157 Lambton Quay
Wellington 6001
New Zealand

State of Nationality

State of Residence

Telephone No.
64 04 499 9058

Facsimile No.
64 04 499 9306

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Catherine Massetti

Telephone No.: (41-22) 338.83.38

003108502

Form PCT/IB/306 (March 1994)

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| | | |
|---|---|---|
| Applicant's or agent's file reference<br>23624 MRB | **FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
| International application No.<br>PCT/NZ 98/00145 | International filing date *(day/month/year)*<br>2 September 1998 | (Earliest) Priority Date *(day/month/year)*<br>26 September 1997 |

Applicant
AUCKLAND UNISERVICES LIMITED

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (See Box II).

4. With regard to the **title**,
☒ the text is approved as submitted by the applicant.
☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
☒ the text is approved as submitted by the applicant
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.
☐ as suggested by the applicant.
☐ because the applicant failed to suggest a figure
☐ because this figure better characterizes the invention
☒ None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ 98/00145

**A. CLASSIFICATION OF SUBJECT MATTER**

Int Cl[6]: A61K 38/17, 38/18

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC6 A61K 38/17, 38/18; IPC5 A61K 37/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN:
AMYLIN, ADRENOMEDULLLIN, BONE, OSTEO, CARTILAGE, CHONDROCYTE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | WO 97/38704, A, (TULANE EDUCATIONAL FUND) 23 October 1997 | 12-27, 31-34, 38-40, 42-49, 51 |
| X | WO 97/07214, A, (US DEPARTMENT OF HEALTH & HUMAN SERVICES) 27 February 1997. Page 6, lines 14-17; | 12-27, 31-34, 38-40, 42-49, 51 |
| X | WO 96/02269, A, (AUCKLAND UNISERVICES LTD) 1 February 1996 | 1-11, 23-30, 34-37, 41, 43-50 |

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 10 MAY 1999 | 17 MAY 1999 |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200<br>WODEN ACT 2606<br>AUSTRALIA<br>Facsimile No.: (02) 6285 3929 | Authorized officer<br><br>D.A. LALLY<br>Telephone No.: (02) 6283 2533 |

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ 98/00145

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 408284, A, (AMYLIN CORPORATION) 16 January 1991 | 1-11, 23-30, 34-37, 41, 43-50 |
| P, X | American Journal of Physiology (1998), Vol. 275 (4, Pt. 1), pp E694-E699, Cornish, J. et al "Systemic administration of amylin increases bone mass, linear growth, and adiposity in adult male mice". Column 2 at E697, line 21 to column 1 at E698, line 9. | 1-11, 23-30, 34-37, 41, 43-50 |
| P,X | American Journal of Physiology (1998), Vol. 274 (5, Pt. 1), pp E827-E833, Cornish, J. et al "Dissociation of the effects of amylin on osteoblast proliferation and bone resorption" | 1-11, 23-30, 34-37, 41, 43-50 |
| X | American Journal of Physiology (1997), Vol. 273 (6, Pt. 1), pp E1113-E1120, Cornish, J. et al "Adrenomedullin is a potent stimulator of osteoblastic activity in vitro and in vivo" | 12-27, 31-34, 38-40, 42-49, 51 |
| X | Principles of Bone Biology (1996), pp 495-505, Reid I.R & Cornish, J. "Amylin and CGRP". Page 497, column 1, line 18 to Page 503, column 1, line 40. | 1-51 |

# INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/NZ 98/00145

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | | Patent Family Member | | | | | |
|---|---|---|---|---|---|---|---|
| WO | 97/38704 | AU | 26730/97 | CA | 2252228 | EP | 904094 |
| | | US | 5888963 | | | | |
| WO | 97/07214 | AU | 67765/96 | CA | 2229741 | EP | 845036 |
| WO | 97/02269 | AU | 29922/95 | | | | |
| EP | 408284 | AT | 137975 | CA | 2020752 | DE | 69026986 |
| | | DK | 401/91 | ES | 2088971 | GB | 8915712 |
| | | GR | 3020680 | IE | 62625 | JP | 4500691 |
| | | SG | 46382 | US | 5405831 | WO | 91/00710 |

END OF ANNEX

09/509,605 17

5630

PATENT COOPERATION TREATY

CORRECTED VERSION

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

11 APR 2000

WIPO

| | | |
|---|---|---|
| Applicant's or agent's file reference 23624MRB/smb | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416). |
| International application No. **PCT/NZ 98/00145** | International filing date *(day/month/year)* 25 September 1998 | Priority Date *(day/month/year)* 26 September 1997 |

International Patent Classification (IPC) or national classification and IPC

**Int. Cl.**[6] A61K 38/17, A61K 38/18

Applicant AUCKLAND UNISERVICES LIMITED

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

| | | |
|---|---|---|
| I | ☒ | Basis of the report |
| II | ☐ | Priority |
| III | ☐ | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| IV | ☐ | Lack of unity of invention |
| V | ☒ | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| VI | ☒ | Certain documents cited |
| VII | ☐ | Certain defects in the international application |
| VIII | ☐ | Certain observations on the international application |

| | |
|---|---|
| Date of submission of the demand 22 April 1999 | Date of completion of the report |
| Name and mailing address of the IPEA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | Authorized Officer<br>**DEBORAH LALLY**<br>Telephone No. (02) 6283 2533 |

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ 98/00145

**I. Basis of the report**

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , filed with the letter of .

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ 98/00145

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Claims **1 to 51** | **YES** |
| | Claims - | **NO** |
| Inventive step (IS) | Claims **1 to 51** | **YES** |
| | Claims - | **NO** |
| Industrial applicability (IA) | Claims **1 to 51** | **YES** |
| | Claims - | **NO** |

2. Citations and explanations (Rule 70.7)

**Document 1**
WO/9707214 US DEPARTMENT OF HEALTH AND HUMAN SERVICES

**Document 2**
WO 96/02269

**Document 3**
EP 408284

**Document 4**
American Journal of Physiology (1996), Vol. 273 (6, Pt.1), pp. E1113-E1120 Cornish, J. *et al* "Adrenomedullin is a potent stimulator of osteoblastic activity *in vitro* and *in vivo*".

**Document 5**
Principles of Bone Biology (1996), pp. 495-505, Reid I.R. & Cornish J. "Amylin and CGRP".

The invention is directed to the use of adrenomedullin and/or amylin to stimulate chondrocyte proliferation. The chondrocyte proliferation is attained by increasing the active concentration of adrenomedullin and/or amylin. This stimulation of chondrocyte proliferation has the subsequent result of stimulating cartilage growth and/or repair *in vivo* or bone growth *in vivo*. The increase in the active concentration of adrenomedullin and/or amylin is attained by the administration of adrenomedullin and/or amylin, or an analog, agonist or fragment of adrenomedullin or amylin

**Document 1**
This document teaches the use of adrenomedullin and/or the antibodies to this peptide to promote bone development. Whereas there is some evidence that adrenomedullin is located proximate to the chondrocytes, but there is only support for the anti-adrenomedullin antibody binding to chondrocytes. Even if the adrenomedullin did bind to chondrocyte receptors, there is no evidence to directly or implicitly suggest that adrenomedullin receptors can be activated by adrenomedullin, and that this activation would result in ensuing proliferation of chondrocytes, rather than some other outcome.. In view of this, claims 1 to 51 are novel and inventive when compared with this document.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ 98/00145

**VI. Certain documents cited**

1. Certain published documents (Rule 70.10)

| Application No. Patent No. | Publication date *(day/month/year)* | Filing date *(day/month/year)* | Priority date ( valid claim) *(day/month/year)* |
|---|---|---|---|
| WO 97/38704 | 23 October 1997 | 17 April 1997 | 18 April 1996 |

2. Non-written disclosures (Rule 70.9)

| Kind of non-written disclosure | Date of non-written disclosure *(day/month/year)* | Date of written disclosure referring to non-written disclosure *(day/month/year)* |
|---|---|---|

**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of : **Box V**

**Document 2**
This document discloses the stimulation of bone growth using amylin or an amylin agonist. The mode of action is posited to be by way of inhibiting osteoclast motility, and by increasing osteoblast proliferation. Unless there is an implicit stimulation of chondrocytes in either of these modes of action or some type of connection between the activities of the different types of cells [osteoblasts, osteoclasts and chondrocytes], it is difficult to conclude that this document either directly teaches or suggests the use of amylin or its agonists to stimulate bone growth by way of chondrocyte proliferation. Thus the invention claimed is novel and inventive over this document.

**Document 3**
This document discloses the treatment of bone disorders using amylin or an amylin agonist. The mode of action is posited to be by way of inhibition/regulation of osteoclastic bone resorption. Unless there is an implicit stimulation of chondrocytes in this mode of action or some type of connection between the activities of the different types of cells [osteoclasts and chondrocytes], it is difficult to conclude that this document either directly teaches or suggests the use of amylin or its agonists to stimulate bone growth by way of chondrocyte proliferation. Thus the invention claimed is novel and inventive over this document.

**Document 4**
This document discloses the use of adrenomedullin to stimulate osteoblast proliferation *in vitro* and to inhibit bone resorbtion by action on the osteoclast calcitonin receptor. Unless there is an implicit stimulation of chondrocytes in this mode of action or some type of connection between the activities of the different types of cells [osteoblasts, osteoclasts and chondrocytes], it is difficult to conclude that this document either directly teaches or suggests the use of adrenomedullin or its agonists to stimulate bone growth by way of chondrocyte proliferation. Thus the invention claimed is novel and inventive over this document.

**Document 5**
This document discloses the use of amylin. The mode of action posited to be by way of reduction/regulation of osteoclastic bone resorbtion, and includes both basal and parathyroid hormone-stimulated bone resorbtion. Amylin is also suggested to stimulate/increase osteoblast proliferation. The document makes no comment upon the effects on chondrocytes, and unless there is an implicit stimulation of chondrocytes in this mode of action or some type of connection between the activities of the different types of cells [osteoblasts, osteoclasts and chondrocytes], it is difficult to conclude that this document either directly teaches or suggests the use of amylin or its agonists to stimulate bone growth by way of chondrocyte proliferation. Thus the invention claimed is novel and inventive over this document.

**INDUSTRIAL APPLICABILITY:**
Claims 1 to 51 are subject matter of Rule 67.1 (methods of treatment of human beings ...) and as such do not require IPE consideration. However, as their subject matter does not contravene Australian law, these claims have been considered.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/17, 38/18 — A3

(11) International Publication Number: WO 99/16406

(43) International Publication Date: 8 April 1999 (08.04.99)

(21) International Application Number: PCT/NZ98/00145

(22) International Filing Date: 25 September 1998 (25.09.98)

(30) Priority Data:
328853 26 September 1997 (26.09.97) NZ

(71) Applicant (*for all designated States except US*): AUCKLAND UNISERVICES LIMITED [NZ/NZ]; UniServices House, 58 Symonds Street, Auckland (NZ).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): REID, Ian, Reginald [NZ/NZ]; 7 Maybeck Road, Mount Albert, Auckland (NZ). CORNISH, Jillian [NZ/NZ]; 22A Godden Crescent, Mission Bay, Auckland (NZ).

(74) Agents: BENNETT, Michael, Roy et al.; Russell McVeagh West–Walker, The Todd Building, Level 5, 171–177 Lambton Quay, Wellington 6001 (NZ).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**

*With international search report.*

*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(88) Date of publication of the international search report: 8 July 1999 (08.07.99)

(54) Title: THERAPEUTIC METHOD

(57) Abstract

This invention is directed to new therapeutic uses which involve the stimulation of chondrocyte proliferation. More particularly, it is directed to the use of amylin and adrenomedullin as agents which stimulate chondrocyte proliferation and which therefore have utility in the treatment of cartilage disorders and/or cartilage mediated bone growth.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ 98/00145

**A. CLASSIFICATION OF SUBJECT MATTER**

Int Cl[6]: A61K 38/17, 38/18

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC6 A61K 38/17, 38/18; IPC5 A61K 37/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN:
AMYLIN, ADRENOMEDULLLIN, BONE, OSTEO, CARTILAGE, CHONDROCYTE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | WO 97/38704, A, (TULANE EDUCATIONAL FUND) 23 October 1997 | 12-27, 31-34, 38-40, 42-49, 51 |
| X | WO 97/07214, A, (US DEPARTMENT OF HEALTH & HUMAN SERVICES) 27 February 1997. Page 6, lines 14-17; | 12-27, 31-34, 38-40, 42-49, 51 |
| X | WO 96/02269, A, (AUCKLAND UNISERVICES LTD) 1 February 1996 | 1-11, 23-30, 34-37, 41, 43-50 |

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ 98/00145

C (Continuation). **DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 408284, A, (AMYLIN CORPORATION) 16 January 1991 | 1-11, 23-30, 34-37, 41, 43-50 |
| P, X | American Journal of Physiology (1998), Vol. 275 (4, Pt. 1), pp E694-E699, Cornish, J. et al "Systemic administration of amylin increases bone mass, linear growth, and adiposity in adult male mice". Column 2 at E697, line 21 to column 1 at E698, line 9. | 1-11, 23-30, 34-37, 41, 43-50 |
| P,X | American Journal of Physiology (1998), Vol. 274 (5, Pt. 1), pp E827-E833, Cornish, J. et al "Dissociation of the effects of amylin on osteoblast proliferation and bone resorption" | 1-11, 23-30, 34-37, 41, 43-50 |
| X | American Journal of Physiology (1997), Vol. 273 (6, Pt. 1), pp E1113-E1120, Cornish, J. et al "Adrenomedullin is a potent stimulator of osteoblastic activity in vitro and in vivo" | 12-27, 31-34, 38-40, 42-49, 51 |
| X | Principles of Bone Biology (1996), pp 495-505, Reid I.R & Cornish, J. "Amylin and CGRP". Page 497, column 1, line 18 to Page 503, column 1, line 40. | 1-51 |

# INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
**PCT/NZ 98/00145**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | | Patent Family Member | | | | |
|---|---|---|---|---|---|---|
| WO | 97/38704 | AU | 26730/97 | CA | 2252228 | EP 904094 |
| | | US | 5888963 | | | |
| WO | 97/07214 | AU | 67765/96 | CA | 2229741 | EP 845036 |
| WO | 97/02269 | AU | 29922/95 | | | |
| EP | 408284 | AT | 137975 | CA | 2020752 | DE 69026986 |
| | | DK | 401/91 | ES | 2088971 | GB 8915712 |
| | | GR | 3020680 | IE | 62625 | JP 4500691 |
| | | SG | 46382 | US | 5405831 | WO 91/00710 |

END OF ANNEX

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(54) Title: THERAPEUTIC METHOD

(57) Abstract

This invention is directed to new therapeutic uses which involve the stimulation of chondrocyte proliferation. More particularly, it is directed to the use of amylin and adrenomedullin as agents which stimulate chondrocyte proliferation and which therefore have utility in the treatment of cartilage disorders and/or cartilage mediated bone growth.

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# THERAPEUTIC METHOD

This invention is directed to new therapeutic uses which involve the stimulation of chondrocyte proliferation. More particularly, it is directed to the use of amylin and adrenomedullin as agents which stimulate chondrocyte proliferation and which therefore have utility in the treatment of cartilage disorders and/or cartilage mediated bone growth.

## BACKGROUND

Amylin is a 37-amino acid peptide cosecreted with insulin from the beta cells of the pancreatic islets. It was first reported by Cooper *et al* in Proceedings of the National Academy of Sciences, USA 84, 8628 (1987) and is the subject of European Patent 289287. Amylin has the following peptide sequence:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-
1 5 10

Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-
11 15 20

Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-
21 25 20

Asn-Val-Gly-Ser-Asn-Thr-Tyr
31 35

The native molecule contains a disulphide bridge between the cysteine residues shown at positions 2 and 7 in the primary structure, is amidated at its COOH-terminus, and is formed as a propeptide.

European Patent 289287 reports a number of biological effects including enhancement of hepatic glucose output, increased production of lactate from skeletal muscle and reduced action of insulin in skeletal muscle.

Amylin is also reported in European Patent 408284 as having value for treatment of bone disorders and calcium imbalance. The patent specification attributes the activity of amylin to an inhibition of osteoclast motility. It is also reported in WO 96/02269 as stimulating bone growth through stimulating osteoblast proliferation.

Adrenomedullin is a 52-amino acid peptide first described in 1993 by Kitamura *et al* (Kitamura, K., *et al.* Adrenomedullin, a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem. Biophys. Res. Commun.* 192:553-560 (1993)). It is present in normal adrenal/medulla and in many other tissues including the atria, ventricles, endothelial cells, lungs, brain, kidneys and bone.

Adrenomedullin shows approximately 20% sequence identity with amylin and can therefore be termed a related peptide (Muff, R., *et al.* Calcitonin, calcitonin gene-related peptide, adrenomedullin and amylin: homologous peptides, separate receptors and overlapping biological actions. *Eur. J. Endocrinol.* 133:17-20 (1995)). Both peptides have an $NH_2$ terminal ring created by a disulphide bond and are amidated at their COOH terminals.

Like amylin, adrenomedullin is also reported to have a range of activities. It is a potent vasodilator. It also has value in the treatment of bone disorders. This is primarily through an ability to stimulate osetoblast activity and proliferation *in vitro* and *in vivo* (Cornish, J., *et al.* Adrenomedullin is a potent stimulator of osteoblastic activity *in vitro* and *in vivo*. *Am. J. Physiol (Endocrinol Metab)* 36:E1113-E1120, (1997)).

However, to date, there has been no report of either of the peptides amylin or adrenomedullin, as having any effect on chondrocytes. It is the applicants finding that both of these peptides are effective in the stimulation of chondrocyte proliferation and therefore on the growth of both cartilage and lineal bone. This effect is believed to be mediated through a single receptor on chrondrocytes which underlies the applicant's invention.

## SUMMARY OF THE INVENTION

The invention has a number of aspects. In a first aspect, the invention provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of increasing the active concentration of amylin within said patient.

Another aspect provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of administering to said patient amylin or an analog thereof in an amount effective to stimulate chondrocyte proliferation.

In another embodiment, the invention provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of increasing the active concentration of adrenomedullin within said patient.

In a further embodiment, the invention provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of administering to said patient adrenomedullin or an analog thereof in an amount effective to stimule chondrocyte proliferation.

In still a further aspect, the invention provides a method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of activating the receptor localised on chondrocytes of said patient to which amylin and/or adrenomedullin bind.

Most preferably, the method involves activation of the adrenomedullin receptor.

Conveniently, in each of the above methods the stimulation of chondrocyte proliferation is part of a method of treating a patient to stimulate cartilage growth and/or repair or to stimulate bone growth.

The invention also provides a method of stimulating chondrocyte proliferation *in vitro* which comprises administering an amount of amylin, adrenomedullin or an analog of either amylin or adrenomedullin to said chondrocytes which is effective in inducing chondrocyte proliferation.

Other aspects include:

the use of amylin or an analog thereof in the preparation of a medicament for effecting chondrocyte proliferation;

the use of adrenomedullin or an analog thereof in the preparation of a medicament for effecting chondrocyte proliferation;

the use of a ligand which binds to and activates the receptor to which amylin and/or adrenomedullin binds (preferably the adrenomedullin receptor) in the preparation of a medicament for effecting chondrocyte proliferation;

the use of an amylin agonist in the preparation of a medicament for effecting chondrocyte proliferation;

the use of an adrenomedullin agonist in the preparation of a medicament for effecting chondrocyte proliferation;

the use of amylin-(1-8) in the preparation of a medicament for effecting chondrocyte proliferation; and

the use of adrenomedullin-(27-52) in the preparation of a medicament for effecting chondrocyte proliferation.

**DESCRIPTION OF THE DRAWINGS**

The present invention is broadly as defined above. However, it will be appreciated by those persons skilled in the art that it is not limited thereto and that it also includes embodiments which are more particularly described below and illustrated by the experimental data presented. This data includes the information shown in the accompanying drawings in which:

Figure 1 shows the effects of daily systemic administration of amylin for 4 weeks on growth plate width in the tibiae of normal adult male mice. n = 20 in each group. *, significantly different from control, P = 0.0002;

Figure 2 shows the effects of daily systemic administration of amylin for 4 weeks on bone length in the tibiae of normal adult male mice. n = 20 in each group. *, significantly different from control, P = 0.004;

Figure 3 shows the effect of the amylin fragment (amylin (1-8)) on epiphyseal growth plate width; and

Figure 4 shows the effect of the adrenomedullin fragment (adm 27-52) on epiphyseal growth plate width.

## DESCRIPTION OF THE INVENTION

As broadly defined above, the present invention relates primarily to methods for stimulating chondrocyte proliferation. The invention therefore has utility in any application where stimulation of chondrocyte proliferation or growth is viewed as desirable, including for example cartilage growth and bone growth.

The applicants have found that chondrocyte proliferation is able to be effected using a number of related approaches. A first such approach is through a focus upon amylin. The applicants have found that increasing the effective concentration of amylin within a patient able to interact with the patients chondrocytes has the effect of stimulating chondrocyte proliferation.

Amylin for use in accordance with this approach can be obtained from any convenient commercial source (such as Bachem California, Torrence, CA, USA). Alternatively, amylin can be synthesised, using the procedure as described by way of example in EP 408284.

The amylin used can be homologous or heterologous to the patient to be treated. For example, amylin from humans and other mammals eg. rat, monkey, dog, cat, mouse, guinea pig, hamster, degus, rabbit and hare can be used. The structure of

these various peptides is reported in *Endocrine Reviews* 1994, 15(2) 163 by Garth J S Cooper which is incorporated herein by reference.

Most conveniently, the effective concentration of amylin will be increased through direct administration using either amylin itself or an amylin pro-drug (a form which is cleaved within the body to release amylin). It is however not the applicants intention to exclude increasing amylin concentration through administration of either amylin agonists (substances which effect a direct increase in the production or activity of amylin within the body, or inhibitors of amylin antagonists (substances which bind amylin or otherwise prevent or reduce the action of amylin within the body. These latter compounds exert an indirect effect on effective amylin concentrations through the removal of an inhibitory mechanism.

Another possibility is administration of a replicable vehicle encoding amylin to the patient. Such a vehicle (which may be a modified cell line or virus which expresses amylin within the patient) could have application in increasing the concentration of amylin within the patient for a prolonged period.

It is also contemplated that amylin analogs can be employed in this invention. As used herein "analog" means a protein which is a variant of another protein through insertion, deletion or substitution of one or more amino acids but which retains at least substantial functional equivalency.

A protein is a functional equivalent of another protein for a specific function if the equivalent protein is immunologically cross-reactive with, and has at least substantially the same function as, the original protein. The equivalent can be, for example, a fragment of the protein, a fusion of the protein with another protein or carrier, or a fusion of a fragment with additional amino acids. For example, it is possible to substitute amino acid in a sequence with equivalent amino acids using conventional techniques. Groups of amino acids normally held to be equivalent are:

(a) Ala, Ser, Thr, Pro, Gly;
(b) Asn, Asp, Glu, Gln;
(c) His, Arg, Lys;
(d) Met, Leu, Ile, Val; and

(e) Phe, Tyr, Trp.

In the case of amylin, the preferred analogs are fragments of the protein. In particular, amylin (1-8) can be used (ie. a fragment consisting of amino acids 1 to 8 of the amylin sequence).

Functional equivalency of analogs can also be readily screened for by reference to the ability of the analog to both bind to and activate the appropriate receptor.

In addition to the above approach, which focuses upon amylin and its analogs, the invention provides a further approach to chondrocyte proliferation. This second approach has a focus upon adrenomedullin. The applicants have found that, in an equivalent manner to amylin, increasing the effective concentration of adrenomedullin within a patient able to interact with the chondrocytes in that patient stimulates chondrocyte proliferation.

For use in this approach, adrenomedullin can be obtained from any convenient commercial source or, as is the case with amylin, synthesised using techniques well known in the art. Such techniques include those described hereinafter.

Again, it is most convenient that the effective concentration of adrenomedullin be increased through direct administration using either adrenomedullin itself or an adrenomedullin pro-drug. However, adrenomedullin agonists or inhibitors of adrenomedullin antagonists are not excluded.

As with amylin, adrenomedullin can also be administered in the form of a replicable vehicle encoding adrenomedullin to the patient for release of adrenomedullin over a prolonged period.

Adrenomedullin analogs can also be employed. For this purpose, the term "analog" has the equivalent meaning of that given above for amylin. In the case of adrenomedullin, a particularly preferred analog is adrenomedullin (27-52) (ie. a fragment consisting of amino acids 27-52 of the adrenomedullin sequence).

The invention still further provides a third approach to chondrocyte proliferation. This further approach focuses upon the receptors on chondrocytes to which amylin

and adrenomedullin bind and upon effecting chondrocyte proliferation through use of any ligand which both binds to and activates these receptors.

It will be appreciated that amylin, analogs of amylin, adrenomedullin and analogs of adrenomedullin are all ligands which achieve this. Indeed, the use of these substances as active agents represents a preferred aspect of the invention. However, it should be appreciated that this approach has not restricted the use of amylin, adrenomedullin and their analogs but also extends to any ligand which fulfils the functional requirement of both binding to and activating (stimulating) the amylin or adrenomedullin receptors. Such additional ligands are, for example, believed to include peptides such as calcitonin gene related peptide (Muff, R., *et al.* Calcitonin, calcitonin gene-related peptide, adrenomedullin and amylin: homologous peptides, separate receptors and overlapping biological actions. *Eur. J. Endocrinol.* 133:17-20 (1995)).

A specific feature of this approach is to employ ligands which bind to and activate the adrenomedullin receptor. This receptor was described in, for example, Kapas, S., *et al.* Cloning and expression of cDNA encoding a rat adrenomedullin receptor. *J. Biol. Chem.* 270:25344-25347 (1995). It is further described in Montuenga, L. M., *et al.* Expression of adrenomedullin and its receptor during embryogenesis suggests autocrine or paracrine modes of action. *Endocrinology* 138:440-451 (1997)).

Additional stimulatory ligands can therefore, for example, be identified by a screening protocol employing at least the ligand binding domain of the adrenomedullin receptor. This screening method can, for example, utilise the expression of the adrenomedullin receptor in Xenopus oocytes using standard recombinant DNA methods and measurement of the adrenomedullin receptor-mediated signal transduction evoked by novel stimulatory ligands.

For therapeutic application, the active compound (amylin, adrenomedullin, analog or ligand) will be formulated as a medicament. The details of the formulation will ultimately depend upon the active compound itself and upon the route of administration chosen. It will however be usual for the medicament to include combination of the active compound with a suitable carrier, vehicle or diluent.

Dosage rates will also be active compound and administration route dependent. However, by way of example, the dosage of active compound to be administered by injection will be in the range of 0.01-100 mg/kg of body weight.

Further, while formulations in which the active compounds represent the sole active principle are most likely to be used, it is by no means intended that formulations which are suitable for combination therapies be excluded. The active compound can be administered together with any other therapeutic agent, including any other agent which has an effect on chondrocyte proliferation.

The invention, in its various aspects, will now be illustrated by the experimental section which follows. It will however be appreciated that the experiments are non-limiting.

## EXPERIMENTAL

### METHODS

**(a) Chondrocyte Monolayer Cell Cultures**

Fresh cartilage samples were collected from the tibial plateaus and femoral condyles of mature, healthy crossbred dogs (2-4 years; 20-25 kg). The chondrocytes were isolated as previously described (Connective Tissue Research 1988; 18:205-222). Briefly, the chondrocytes were obtained by pronase and collagenase digestion of the cartilage, then the cells were centrifuged, washed and resuspended in media before being cultured in 75 $cm^2$ tissue culture flasks. The cells were incubated under 5% $CO_2$ and 95% air at 37°C. Confluence was reached by 7-10 days, at which time the cells were subcultured. After trypsinization, the cells are rinsed and resuspended in fresh medium, then seeded at 5 x $10^4$ cells/ml in 24-well plates (0.5 ml cell suspension per well, ie. 1.4 x $10^4$ cells/$cm^2$). *Proliferation studies* (cell counts and thymidine incorporation) were performed. Subconfluent population were changed to serum-free medium with 0.1% bovine serum albumin plus the experimental compounds. Cell numbers were analysed at 24 hours after addition of the peptide or vehicle. The cell numbers were determined using a haemocytometer chamber. Results were expressed per well. [$^3$H]-*thymidine incorporation* was assessed by pulsing the cells with [$^3$H]-thymidine (1uCi/well) two hours before the end of the experimental incubation. The experiment was terminated at 24 hours by washing

the cells in media containing cold thymidine followed by the addition of 10% tricholoroacetic acid. The precipitate was washed twice with ethanol:ether (3:1) and the wells desiccated at room temperature. The residue was redissolved in 2 M KOH at 55°C for 30 mins, neutralised with 1 M HCl, and an aliquot counted for radioactivity. Results were expressed as dpm per well. For both cell counts and thymidine incorporation, each experiment was performed at least 4 different times using experimental groups consisting of at least 6 wells.

**(b) Chondrocytes 3-dimensional cell cultures in alginate beads**

Alginate head cultures were established as described by Guo, *et al.* Culture and growth characteristics of chondrocytes encapsulated in alginated beads. *Connective Tissue Research.* 19:277-297 (1989). Briefly the cells were suspended in a solution of 1.25% (wt/vol) alginate in HEPES (20 mM HEPES buffer pH neutral) at a density of $2x10^6$ cells/ml> The suspension of chondrocytes were slowly extruded through a 22-gauge needle in a dropwise manner into 40 ml of 0.1 M $CaCl_2$ solution. After instantaneous gelation, the beads were allowed to further polymerise in $CaCl_2$ solution (10 mins, room temperature). The beads were washed sequentially, twice in 0.15 M NaCl and twice in Dulbecco's modified Eagle's medium (DME). After the washing procedure, the beads were placed into 24-well culture plates (10 beads/well) and fed with 1ml 10% fetal calf serum (FCS) SMW with 5µg/ml ascorbic acid. The cultures were maintained at 37°C in a humidified atmosphere of 5% $CO_2$ in air. The medium was changed every second day. On day 4 and 6 of culture, peptide or vehicle was added. Cell numbers were analysed at day 8 by exposing alginate beads to 50 mM ethylenediaminetetraacetic acid (EDTA) for approximately 10 minutes at 37°C. Counting was performed in a haemocytometer chamber. Results were expressed per well. Tritiated-thymidine incorporation ($^3$H-thymidine) was assessed by pulsing the beads with $^3$H-thymidine (1µCi/well) 48 hours before the end of experimental incubation. Experiments were then terminated at day 8 of culture by dissolving the beads in 50 mM EDTA. The cells were washed twice with distilled water by centrifuging. Pellets were resuspended and counted for radioactivity.

**(c) *In Vivo* Study: Experimental Design**

Two groups of 20 sexually mature male Swiss mice aged between 40 and 50 days and weighing 25-32g, were given daily subcutaneous injections (50 ul) in the loose skin at the nape of the neck for 5 days/week over 4 consecutive weeks. The treated

group was injected with peptide at a dose of 300 ug/kg/injection and the control group was injected with vehicle (water). Animals were housed in a room maintained at 20°C on 12-hour light/dark cycles. They were fed diet 86 rodent pellets (New Zealand Stockfeed Ltd) ad libitum throughout the experiment. Each animal's weight was recorded at the beginning and end of the experiment. One day after the last injection, animals were sacrificed by cervical dislocation. They study had the approval of the local institutional review board.

The tibiae were dissected free of adherent tissue. Tibial lengths were recorded by measuring the distance between the proximal epiphysis and the distal tibio-fibular junction using an electronic micrometer (Digimatic Calipers, Mitutoyo, Japan). Bones were placed in 10% phosphate-buffered formalin for 24 hours and then dehydrated in a graded series of ethanol solutions and embedded, undecalcified, in methylmethacrylate resin. Tibiae were sectioned longitudinally through the frontal plane and calvariae were cut cross-sectionally at the base of the parietal bone. All sections were 4 um think and were cut on a Leitz microtome using a tungsten-carbide knife (Microknife Sharpening, Utah, USA). Sections were mounted on gelatin-coated slides and air-dried. They were stained with Goldner's tri-chrome and examined using an Olympus BX 50 microscope (Olympus Optical Co Ltd, Tokyo, Japan) which was attached to an Osteomeasure Image Analyzer (Osteometrics Inc. Atlanta, GA).

Tibial histomorphometric analyses were made from three adjacent sections one third of the way through the anterior/posterior depth of the proximal tibiae. Epiphyseal growth plate thickness was measured at three sites evenly spaced along its length. All measurements were made by one operator who was blinded to the treatment group of each bone.

**Materials**

Rat amylin was sourced from Bachem California, Torrance, CA, USA. Lyophilised material was dissolved in water prior to administration. Methylmethacrylate was purchased from Acros Organics N.V., Geel, Belgium.

Rat amylin-(1-8) used in this study was a COOH-terminal amide synthesized on methylbenzhydrylamine resin by standard solid-phase techniques followed by hydrogen fluoride deprotection and cleavage from the resin. Amylin-(1-8) was

cyclized in a dilute solution of 90% acetic acid by treatment with methanol solutions of iodine and purified to >96% homogeneity by reverse-phase high performance liquid chromatograph (RP HPLC). Structures were confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF system, model G2025 A, Hewlett Packard CA, USA) and amino acid analysis of acid hydrolysates %%%4929%%%.

Human adrenomedullin and its fragments were synthesized on methylbenzhydrylamine resin using standard solid-phase procedures, and cleaved with hydrogen fluoride/anisole. Sequences containing a disulfide bridge were cyclized by titration with $I_2$ in 90% acetic acid/water solutions. Crude materials were purified by gel filtration on Sephadex columns in 50% acetic acid followed by gradient elution on C18 silica using acetonitrile/0.1% trifluoroacetic acid eluants. Homogeneity of final peptides was assessed by thin layer chromatography, analytical HPLC, amino acid analysis and matrix-assisted laser-desorption-ionization mass spectroscopy. Purities were usually >98%.

**Statistical Analysis**

Data are presented as mean ± sem. Where parameters have been measured more than once in each animal these values have been averaged to produce a single value for each animal before further analysis. The significant of treatment effects was evaluated using Student's *t* tests for unpaired data. These comparisons were specified a priori, so adjustment of $\alpha(0.05)$ was not necessary.

## RESULTS

### Amylin

**(a) Chondrocyte Cell Cultures**

Amylin influenced chondrocyte proliferation, increasing cell numbers from 4.12 ± 0.23 ($x10^4$) (mean ± sem) in control cells to 5.11 ± 0.21 ($x10^4$) in those cells incubated with amylin ($p=0.01$) as well as increasing thymidine incorporation (ie. DNA synthesis) from 20725 ± 997 dpm in control cells to 25937 ± 1203 dpm in amylin-treated cells.

**(b) Chondrocytes 3-dimensional cell cultures in alginate beads**

Amylin again influenced chondrocyte proliferation, increasing cell numbers from 5.58 ± 0.16 ($x10^4$) (mean ± sem) in control cells to 6.07 ± 0.05 ($x10^4$) in those cells incubated with amylin ($10^{-10}$M) ($p<0.03$) as well as increasing thymidine incorporation (ie. DNA synthesis) from 1135 ± 85 dpm in control cells to 2584 ±229 dpm in amylin-treated cells ($p<0.001$).

**(c) *In Vivo* Study**

Amylin influenced the tibial growth plate, increasing its width from 0.083 ± 0.005 mm (mean ± sem) in the control animals to 0.108 ± 0.003 mm in those receiving amylin (P = 0.0002) (Figure 1). The total length of the tibiae was also increased from 11.31 ± 0.07 mm in control animals to 11.67 ± 0.09 mm in animals injected with amylin (P-0.004) (Figure 2).

**Amylin 1-8**

(a) Amylin-(1-8) also influenced chondrocyte proliferation, increasing cell numbers from 3.23 ± 0.11 ($x10^4$) (mean ± sem) in control cells to 3.63 ± 0.09 ($x10^4$) in those cells incubated with amylin-(1-8) (10-8M) ($p = 0.02$) as well as increasing thymidine incorporation (DNA synthesis) from 26859 ± 423 dpm in control cells to 28932 ±628 dpm in amylin=(1-8) treated cells ($p = 0.02$).

(c) The growth plate width in the proximal tibiae of mice injected systemically with amylin-(1-8) is significantly increased compared to control animals (mean ± sem: 0.111 mm ± 0.004 compared to 0.081 mm ± 0.004; $p<0.0001$). See Figure 3.

**Adrenomedullin**

(a) Adrenomedullin influenced chondrocyte proliferation, increasing cell numbers from 1.79 ± 0.07 (x $10^4$) (mean ± sem) in control cells to 2.27 ± 0.12 (x $10^4$) in those cells incubated with adenomedullin ($10^{-9}$M) ($p<0.01$).

**Adrenomedullin-(27-52)**

(c) Adrenomedullin-(27-52) increased the growth plate width from 0.094 mm ± 0.003 (mean ± sem) in control animals to 0.11 mm ± 0.003 in adrenomedullin-(27-52) ($p=0.003$). See Figure 4.

**INDUSTRIAL APPLICATION**

The above results clearly show that amylin and its anlogs (amylin-(1-8), for example) has the ability to stimulate chondrocyte proliferation. Similarly, adrenomedullin and its analogs (adrenomedullin-(27-52) have equivalent capability.

The results additionally show the ability of both amylin, adrenomedullin and their analogs to influence the growth of cartilage as well as increased bone growth. This latter effect is consistent with the formation of bone on a template of cartilage tissue.

Both amylin and adrenomedullin are believed to be exerting the effect on chondrocyte proliferation/cartilage growth/bone formation through the mediation of the amylin/adrenomedullin receptor.

The present invention therefore provides new approaches to chondrocyte proliferation. These involve firstly increasing the active concentration of amylin/adrenomedullin in a patient and secondly the activation of the amylin/adrenomedullin receptor localised on chondrocyte cells.

The approaches of the invention have application in the treatment of patients in a variety of conditions. Principal amongst these are conditions where the patient is suffering from a cartilage defect, either through injury or through degenerative, inflammatory or other disease.

The approaches of the invention also have application in the stimulation of bone growth, particularly lineal bone growth. This provides the invention with application in treating patients (for example, children) who are of short stature or who otherwise suffer from defects which would benefit from stimulation of the growth of limb bones.

The invention also has application *in vitro*. Extracted chondrocytes can be proliferated using the present methods. The proliferated chondrocytes can then be employed in methods of therapy, particularly those which involve the treatment of damaged cartilage.

It will be appreciated by those persons skilled in the art that the above description is provided by way of example only and that numerous changes and variations can be made while still being within the scope of the invention as defined by the appended claims.

**CLAIMS**

1. A method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of increasing the active concentration of amylin within said patient.

2. A method of treating a patient to stimulate cartilage growth and/or repair *in vivo* through stimulation of chondrocyte proliferation which comprises the step of increasing the active concentration of amylin within said patient.

3. A method of treating a patient to stimulate bone growth *in vivo* through stimulation of chondrocyte proliferation which comprises the step of increasing the active concentration of amylin within said patient.

4. A method according to any one of claims 1 to 3 wherein the active concentration of amylin is increased through administration of amylin to said patient.

5. A method according to any one of claims 1 to 3 wherein the active concentration of amylin is increased through administration of an amylin agonist.

6. A method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of administering to said patient amylin or an analog thereof in an amount effective to stimulate chondrocyte proliferation.

7. A method of treating a patient to stimulate cartilage growth and/or repair *in vivo* through stimulation of chondrocyte proliferation which comprises the step of administering to said patient amylin or an analog thereof in an amount effective to stimulate chondrocyte proliferation.

8. A method of treating a patient to stimulate bone growth *in vivo* through stimulation of chondrocyte proliferation which comprises the step of administering to said patient amylin or an analog thereof in an amount effective to stimulate chondrocyte proliferation.

9. A method according to any one of claims 6 to 8 wherein amylin is administered to said patient.

10. A method according to any one of claims 6 to 8 wherein an analog of amylin is administered to said patient.

11. A method according to claim 10 wherein said amylin analog is amylin-(1-8).

12. A method of treating a patient to stimulate chondrocyte proliferation *in vitro* which comprises the step of increasing the active concentration of adrenomedullin within said patient.

13. A method of treating a patient to stimulate cartilage growth and/or repair *in vivo* through stimulation of chondrocyte proliferation which comprises the step of increasing the active concentration of adrenomedullin within said patient.

14. A method of treating a patient to stimulate both growth *in vivo* through stimulation of chondrocyte proliferation which comprises the step of increasing the active concentration of adrenomedullin within said patient.

15. A method according to any one of claims 12 to 14 wherein the active concentration of adrenomedullin is increased through administration of adrenomedullin to said patient.

16. A method according to any one of claims 13 to 15 wherein the active concentration of adrenomedullin is increased through administration of an adrenomedullin agonist.

17. A method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of administering to said patient adrenomedullin or an analog thereof in an amount effective to stimule chondrocyte proliferation.

18. A method of treating a patient to stimulate cartilage growth and/or repair *in vivo* through stimulation of chondrocyte proliferation which comprises the step of administering to said patient adrenomedullin or an analog thereof in an amount effective to stimulate chondrocyte proliferation.

19. A method of treating a patient to stimulate bone growth *in vivo* through stimulation of chondrocyte proliferation which comprises the step of

administering to said patient adrenomedullin or an analog thereof in an amount effective to stimulate chondrocyte proliferation.

20. A method according to any one of claims 17 to 19 wherein adrenomedullin is administered to said patient.

21. A method according to any one of claims 17 to 19 wherein an analog of adrenomedullin is administered to said patient.

22. A method according to any one of claims 17 to 19 wherein said adrenomedullin analog is adrenomedullin-(27-52).

23. A method of treating a patient to stimulate chondrocyte proliferation *in vivo* which comprises the step of activating a receptor localised on chondrocytes of said patient to which amylin and/or adrenomedullin binds.

24. A method of treating a patient to stimulate cartilage growth and/or repair *in vivo* through stimulation of chondrocyte proliferation which comprises the step of activating a receptor localised on chondrocytes of said patient to which amylin and/or adrenomedullin binds.

25. A method of treating a patient to stimulate bone growth *in vivo* through stimulation of chondrocyte proliferation which comprises the step of activating a receptor localised on chondrocytes of said patient to which amylin and/or adrenomedullin binds.

26. A method according to any one of claims 23 to 25 wherein the receptor which is activated is the adrenomedullin (ADM) receptor.

27. A method according to any one of claims 23 to 26 wherein receptor activation is effected through administration of a ligand which binds to and activates the receptor.

28. A method according to any one of claims 23 to 26 wherein receptor activation is effected through administration of amylin.

29. A method according to any one of claims 23 to 26 wherein receptor activation is effected through administration of an amylin analog.

30. A method according to claim 29 wherein the amylin analog is amylin-(1-8).

31. A method according to any one of claims 23 to 26 wherein ADM receptor activation is effected through administration of adrenomedullin.

32. A method according to any one of claims 23 to 26 wherein receptor activation is effected through administration of an adrenomedullin analog.

33. A method according to claim 32 wherein the adrenomedullin analog is adrenomedullin-(27-52).

34. A method of stimulating chondrocyte proliferation *in vitro* which comprises administering an amount of amylin, adrenomedullin or an analog of either amylin or adrenomedullin to said chondrocytes which is effective in inducing chondrocyte proliferation.

35. A method according to claim 34 wherein an effective amount of amylin is administered.

36. A method according to claim 34 wherein an effective amount of an amylin analog is administered.

37. A method according to claim 36 wherein the amylin analog is amylin-1-8.

38. A method according to claim 34 wherein an effective amount of adrenomedullin is administered.

39. A method according to claim 34 wherein an effective amount of an adrenomedullin analog is administered.

40. A method according to claim 39 wherein the adrenomedullin analog is adrenomedullin-27-52.

41. The use of amylin or an analog thereof in the preparation of a medicament for effecting chondrocyte proliferation.

42. The use of adrenomedullin or an analog thereof in the preparation of a medicament for effecting chondrocyte proliferation.

43. The use of a ligand which binds to and activates a receptor localised on chondrocytes to which amylin and/or adrenomedullin binds in the preparation of a medicament for effecting chondrocyte proliferation.

44. The use of claim 43 wherein the ligand is one which binds to and activates the adrenomedullin (ADM) receptor.

45. The use of any one of claims 41 to 44 wherein the medicament is for the growth and/or repair of cartilage.

46. The use of any one of claims 41 to 44 wherein the medicament is for the growth of bone.

47. The use of claim 46 wherein the medicament is for effecting the lineal growth of bone.

48. The use of an amylin agonist in the preparation of a medicament for effecting chondrocyte proliferation.

49. The use of an adrenomedullin agonist in the preparation of a medicament for effecting chondrocyte proliferation.

50. The use of amylin-(1-8) in the preparation of a medicament for effecting chondrocyte proliferation.

51. The use of adrenomedullin-(27-52) in the preparation of a medicament for effecting chondrocyte proliferation.

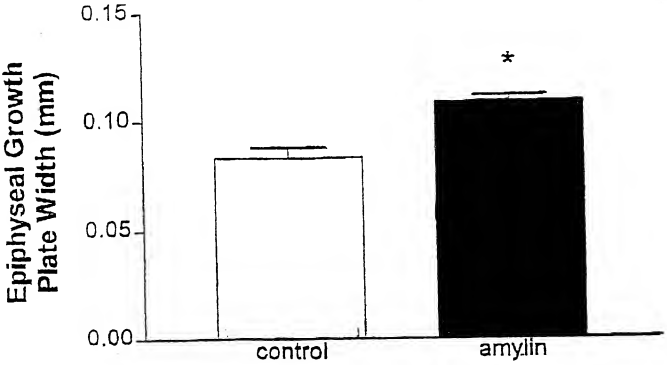


FIGURE 1

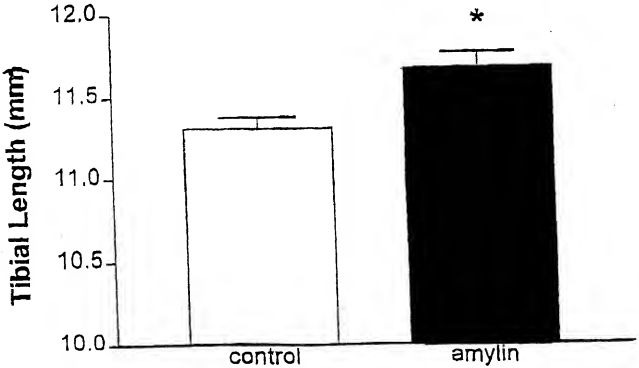


FIGURE 2

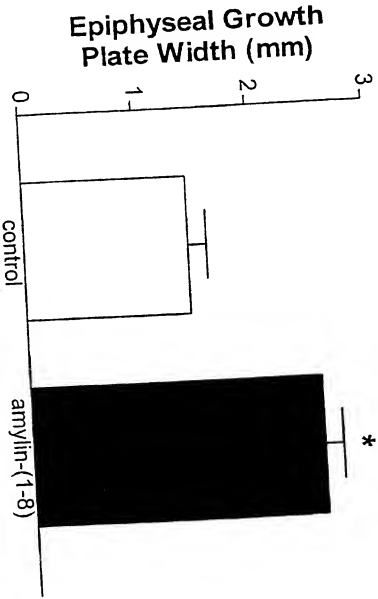


FIGURE 3

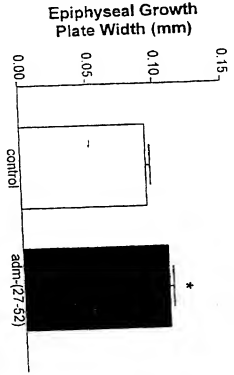
Epiphyseal Growth
Plate Width (mm)
0.00
0.05
0.10
0.15
control
adm-(27-52)
*

FIGURE 4